Autonomic Neuroscience: Basic and Clinical 193 (2015) 44–50

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

The medial amygdaloid nucleus modulates the baroreflex activity in conscious rats

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ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 13 July 2015
Accepted 14 July 2015

Keywords:
Medial amygdaloid nucleus
Baroreflex
$CoCl_2$
Cardiovascular system

ABSTRACT

The medial amygdaloid nucleus (MeA) is involved in cardiovascular control. In the present study we report the effect of MeA pharmacological ablations caused by bilateral microinjections of the nonselective synaptic blocker $CoCl_2$ on cardiac baroreflex responses in rats. MeA synaptic inhibition evoked by local bilateral microinjection of 100 nL of $CoCl_2$ (1 mM) did not affect blood pressure or heart rate baseline, suggesting no tonic MeA influence on resting cardiovascular parameters. However, 10 min after $CoCl_2$ microinjection into the MeA of male Wistar rats, the reflex bradycardic response evoked by intravenous infusion of phenylephrine was significantly enhanced when compared with the reflex bradycardic response observed before $CoCl_2$. The treatment did not affect the tachycardic responses to the intravenous infusion of sodium nitroprusside (SNP). Baroreflex activity returned to control values 60 min after $CoCl_2$ microinjections, confirming a reversible blockade. The present results indicate an involvement of the MeA in baroreflex modulation, suggesting that synapses in the MeA have an inhibitory influence on the bradycardic component of the baroreflex in conscious rats.

1. Introduction

The amygdaloid complex is a limbic structure associated with autonomic, neuroendocrine and behavioral responses (Fortaleza et al., 2011; Ma and Morilak, 2005; Price et al., 1987; Sah et al., 2003) that is located in the temporal lobe between the external capsule and the hypothalamus. Based on its cytoarchitecture and chemoarchitecture, it has been divided into subnuclei having extensive internuclear and intranuclear connections (Krettek and Price, 1978b; Price et al., 1987). The amygdaloid complex is divided into three groups: (1) the deep or basolateral group, which includes the lateral, basal and the accessory basal nucleus; (2) the superficial or cortical-like group, which includes the cortical nucleus and the nucleus of the lateral olfactory tract; and (3) the centromedial group, composed by the medial and central nuclei (Pitkanen et al., 1997; Sah et al., 2003).

Among the amygdaloid complex nuclei, the medial amygdaloid nucleus (MeA) is involved in cardiovascular control and modulates stress responses (Fortaleza et al., 2009, 2011, 2012b; Gelsema et al., 1987; Kubo et al., 2004; Morilak et al., 2005).

Electrical stimulation of the MeA has been reported to evoke mean arterial pressure (MAP) and heart rate (HR) increases in anesthetized rats (Faiers et al., 1975). In addition, we have previously shown that microinjection of noradrenaline into the MeA caused cardiovascular changes, and that stress-evoked heart rate increases are modulated by different types of adrenoceptors in the MeA (Fortaleza et al., 2011, 2012b), suggesting its involvement in central cardiovascular control.

Baroreceptor activity provides an essential feedback to the central nervous system (CNS) for moment-to-moment control of the cardiovascular function, in order to maintain arterial pressure within a narrow functional range (Michelini, 1994; Sved and Gordon, 1994). Moreover, it has been proposed that a resetting of baroreflex activity towards higher blood pressure values mediates, at least in part, autonomic and cardiovascular changes during physical exercise and stress situations (Crestani et al., 2010b; Dampney et al., 2008; DiCarlo and Bishop, 1992). Defensive reactions are associated with changes in cardiovascular activity. Cardiovascular changes include increases in MAP, HR, sympathetic nerve activity and skeletal muscle blood flow (Coote et al., 1979). These simultaneous enhancements in MAP, HR, and sympathetic vasomotor activity imply in a reset of the baroreceptor reflex (Coote et al., 1979; Hatton et al., 1997; Hilton and Zbrozyna, 1963; Nunomura et al., 1983; Porter, 2000; Schlor et al., 1984; Turnbull et al., 1993).

The MeA is connected with medullary structures that appear to be the primary site involved in baroreflex responses (Dampney, 1994; Krettek and Price, 1978a; Michelini, 1994; Schreihofer and Guyenet, 2002; Sved and Gordon, 1994). Moreover, it sends projections to several limbic structures that are involved in baroreflex modulation, such as the medial prefrontal cortex (Resstel and Correa, 2006; Sevoz-Couche et al., 2006), the bed nucleus of the stria terminalis (Crestani et al., 2006), the

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http://dx.doi.org/10.1016/j.autneu.2015.07.003
1566-0702/

periaqueductal grey area (Pelosi et al., 2007), the hypothalamus (Crestani et al., 2010a; Sevoz-Couche et al., 2006), lateral septal area (Scopinho et al., 2007, 2012) and the diagonal band of Broca (Crestani et al., 2008b).

There is functional evidence showing a possible MeA involvement in the basal control of baroreflex activity, resulting from power spectral analysis that evaluates variability of the HR and MAP (Neckel et al., 2012; Quagliotto et al., 2008), or in BPH/2J mice, a neurogenic model of hypertension, in which the MeA was proposed to participate in cardiac baroreflex sensitivity control (Jackson et al., 2014), consequently it would be relevant to evaluate the role of MeA in the modulation on baroreflex activity response evoked by intravenous infusion of phenylephrine (Phe) or sodium nitroprusside (SNP) in conscious rats.

The hypothesis of the present study is that the MeA is involved in baroreflex modulation in conscious rats. To investigate this hypothesis, we analyzed the effect of the blockade of synaptic transmission in the MeA, caused by local microinjection of $CoCl_2$ that reduces pre-synaptic Ca^{2+} influx leading to inhibition of neurotransmitter release, on the cardiac baroreflex response evoked by the intravenous infusion of either Phe or SNP.

2. Material and methods

2.1. Animal preparation

Twenty male Wistar rats weighing 230–270 g were used. Animals were kept in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Rats were housed individually in plastic cages under standard laboratory conditions; a 12 h light–dark cycle, and free access to food and water. Housing conditions and experimental procedures were approved by the Institution's Animal Ethical Committee (Proc 128/2010).

Four days before experiments, rats were anesthetized with tribromoethanol (250 mg/kg i.p.). After scalp anesthesia with 2% lidocaine, the skull was exposed and stainless steel guide cannulas (26G) were stereotaxically implanted bilaterally into the MeA. Coordinates for cannula implantation into the MeA were: AP = +6.2 mm; L = +3.4 mm from the medial suture and V = −8.8 mm, based on the rat brain atlas of Paxinos and Watson (1997). One day before the experiment, rats were anesthetized with tribromoethanol and a catheter was inserted into the abdominal aorta through the femoral artery for MAP and HR recording. A second catheter was implanted into the femoral vein for infusion of either phenylephrine or SNP. Catheters were tunneled under the skin and exteriorized on the animal's dorsum.

2.2. Measurement of cardiovascular responses

Experiments were carried out between 08:00–12:00 h. On the day of the experiment, animals were allowed a 15 min period to adapt to experimental room's conditions, such as sound and illumination, before starting blood pressure and HR recording. Another 15 min period was allowed before beginning the experiments. The room was acoustically isolated and had constant background noise provided by an air exhauster. Care was taken to start injections when stable blood pressure and specially heart rate recordings were observed. The injection needle was slowly introduced into the guide cannula without touching or restraining the animals. Pulsatile arterial pressure of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, Palo Alto, Califórnia, USA) and an acquisition board (MP100A, Biopac Systems Inc, Santa Barbara, Califórnia, USA) connected to a computer. MAP and HR values were derived from the blood pressure recordings and processed online.

2.3. Drug injection

Drugs were dissolved in artificial cerebrospinal fluid (aCSF) with the following composition: NaCl 100 mM; Na_3PO_4 2 mM; KCl 2,5 mM; $MgCl_2$ 1,0 mM; $NaHCO_3$ 27 mM; $CaCl_2$ 2,5 mM (pH = 7.4). Needles (33G, Small Parts, Miami Lakes, Flórida, USA) that were used for microinjection into the MeA were 1 mm longer than guide cannulas and were connected to a 1 μL syringe (7002H, Hamilton, USA) through PE-10 tubing. $CoCl_2$ or vehicle was injected in a final volume of 100 nL in each side of the MeA. After a 20 s period, the needle was removed and inserted into the second guide cannula for microinjections into the contralateral side.

2.4. Baroreflex stimulation

Baroreflex was activated by intravenous infusion of phenylephrine (Phe, 50 μg/kg; 0.34 mL/min) or SNP (50 μg/kg; 0.8 mL/min), using an infusion pump (K.D. Scientific, Holliston, Massachusetts, USA).

2.5. Method of baroreflex evaluation

HR values matching MAP variations were determined. Paired values of MAP and HR variations, evoked by Phe or SNP, were plotted to generate sigmoid curves (Head & McCarty, 1987) for each rat, which were used to determine baroreflex activity. Commercial software (Prism, Prism, GraphPad, San Diego, California, USA) was used to generate curves.

To study bradycardic and tachycardic responses separately, HR values matching 10, 20, 30 and 40 mm Hg MAP changes were calculated. Values were plotted to create linear regression curves for each rat (Crestani et al., 2006; Resstel et al., 2004) and their slopes were compared to verify changes in baroreflex gain. The delay in reflex bradycardia and tachycardia was about 1.2 s because of the time of baroreflex synapse processing that is 700 ms according to Su et al. (1992) and the integration factor of the recording system that was about 500 ms.

2.6. Drugs

The following drugs were used: phenylephrine-HCl; CoCl2 (Sigma, St. Louis, Missouri, USA); sodium nitroprusside; urethane (Sigma, St Louis, Missouri, USA) and tribromoethanol (Aldrich, St Louis, Missouri, USA).

2.7. Experimental protocols

All animals used in this study received three infusions of either phenylephrine or SNP to determine control baroreflex response. One group received bilateral microinjections of aCSF into the MeA and infusions of phenylephrine or sodium nitroprusside were repeated 10 min after this procedure. A second group received bilateral microinjections of $CoCl_2$ (1 mM; Crestani et al., 2006) into the MeA, and phenylephrine or nitroprusside infusions were repeated 10 and 60 min after injection. A third group received bilateral microinjections of $CoCl_2$ (1 mM) into structures surrounding the MeA, and phenylephrine or nitroprusside infusions were repeated 10 min after injection. The order of infusions was randomized. $CoCl_2$ interferes with synaptic Ca^{2+} and consequently causes nonspecific synaptic blockade. Its effectiveness is accepted as indicative of synaptic involvement (Kretz, 1984).

2.8. Histological procedure

At the end of the experiments, rats were anesthetized with urethane (1.25 g/kg, i.p.) and 100 nL of 1% Evan's blue dye was bilaterally injected into the MeA to label injection sites. The chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain perfused with 10% formalin through the left heart ventricle. Brains

were postfixed for 24 h at 4 °C, and 40 μm sections were cut using a cryostat (CM-1900, Leica, Germany). Sections were stained with 1% cresyl violet and injection sites were identified.

2.9. Statistical analysis

Baseline cardiovascular values before and after $CoCl_2$ or aCSF were compared using the Student's t test. Baroreflex was analyzed using sigmoid curves that were characterized by 4 parameters: (i) P1 (bpm) lower HR plateau and P2 (bpm) upper plateau; (ii) HR range (bpm), i.e. difference between upper and lower plateau levels; (iii) median blood pressure (BP_{50}, mm Hg) which is the MAP at 50% of the HR range; and (iv) average gain (G, bpm/mm Hg) which is the average slope of the curves (Korner et al., 1972; Head & McCarty, 1987). Significant differences between sigmoid curves or linear regressions parameters to $CoCl_2$ microinjection into the MeA were analyzed using one-way ANOVA followed by the Dunnett's test. Significant differences between sigmoid curve and linear regressions parameters to aCSF microinjection into the MeA or $CoCl_2$ microinjection into structures surrounding the MeA were analyzed using the Student's t test. It was assumed $P < 0.05$ as significant.

3. Results

Fig. 1 shows a representative photomicrograph of a MeA coronal section and Fig. 2 shows a diagrammatic representation, according (Paxinos and Watson, 1997) of the all microinjection sites from experimental groups.

3.1. Effect of bilateral microinjections of aCSF into the MeA on the baroreflex response to intravenous infusion of phenylephrine or sodium nitroprusside in conscious rats

Bilateral microinjections of 100 nL of aCSF (n = 7) into the MeA did not affect MAP (97 ± 5 vs 99 ± 4 mm Hg, $t = 0.05$, $P > 0.05$) or HR (340 ± 11 vs 345 ± 12 bpm, $t = 0.03$, $P > 0.05$) baseline.

Nonlinear and linear regression analysis of baroreflex activity indicated that microinjections of aCSF into the MeA did not affect the baroreflex response (Fig. 3). The nonlinear regression analysis indicated that aCSF had no effect on the baroreflex parameters analyzed in the sigmoid curve (Fig. 3 and Table 1). Moreover, linear regression also indicated no differences in the slope of bradycardic (−1.70 ± 0.22 vs −1.59 ± 0.20 bpm/mm Hg, $t = 0.3$, $P > 0.05$) or tachycardic (−1.66 ± 0.18 vs −1.84 ± 0.11 bpm/mm Hg, $t = 0.9$, $P > 0.05$) responses after aCSF microinjection into the MeA (Fig. 3).

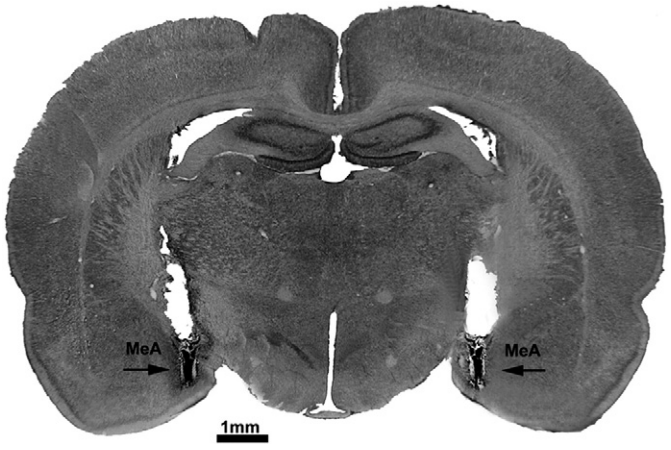

Fig. 1. Photomicrograph of a coronal brain section from one representative rat, showing the microinjection site in the medial amygdaloid nucleus (MeA).

3.2. Effect of bilateral microinjections of $CoCl_2$ (1 mM) in the structures surrounding the MeA on the baroreflex response to intravenous infusion of phenylephrine or sodium nitroprusside in conscious rats

Bilateral microinjections of 100 nL of 1 mM $CoCl_2$ (n = 5) in structures surrounding the MeA did not affect MAP (97 ± 5 vs 100 ± 7 mm Hg, $t = 0.08$, $P > 0.05$) or HR (353 ± 10 vs 358 ± 13 bpm, $t = 0.08$, $P > 0.05$) baseline.

Nonlinear and linear regression analysis of baroreflex activity indicated that microinjections of 1 mM $CoCl_2$ in structures surrounding the MeA (n = 5) did not affect the baroreflex response (Fig. 4). The nonlinear regression analysis indicated that the 1 mM $CoCl_2$ had no effect on the baroreflex parameters analyzed in the sigmoid curve (Fig. 4 and Table 1). Moreover, the linear regression also indicated there are no differences in the slope of bradycardic (−1.91 ± 0.13 vs −1.64 ± 0.23 bpm/mm Hg, $t = 0.8$, $P > 0.05$) or tachycardic (−1.72 ± 0.20 vs −1.91 ± 0.10 bpm/mm Hg, $t = 0.8$, $P > 0.05$) responses after 1 mM $CoCl_2$ in structures surrounding the MeA (Fig. 4).

3.3. Effect of bilateral microinjections of $CoCl_2$ (1 mM) into the MeA on the baroreflex response to intravenous infusion of phenylephrine or sodium nitroprusside in conscious rats

Bilateral microinjections of 100 nL of 1 mM $CoCl_2$ (n = 7) into the MeA did not affect MAP (99 ± 4 vs 101 ± 5 mmHg, $t = 0.09$, $P > 0.05$) or HR (348 ± 12 vs 354 ± 10 bpm, $t = 0.07$, $P > 0.05$) baseline.

Nonlinear and linear regression analysis of baroreflex activity indicated that microinjection of 1 mM $CoCl_2$ into the MeA altered the baroreflex response (Fig. 5). The nonlinear regression analysis indicated that 1 mM $CoCl_2$ into the MeA only increased the curve slope (Fig. 5 and Table 1). Moreover, linear regression indicated that the slope of the bradycardic (−1.21 ± 0.23 vs −1.95 ± 0.20 bpm/mm Hg, $F_{(2,20)} = 3.66$, $P < 0.05$) response was increased after microinjection of 1 mM $CoCl_2$ into the MeA, but the tachycardic (−2.31 ± 0.30 vs −2.04 ± 0.35 bpm/mm Hg, $F_{(2,20)} = 0.19$, $P > 0.05$) response was not altered after MeA blockade (Fig. 5). The recovery of baroreflex to control levels was observed 60 min after the administration of 1 mM $CoCl_2$ into the MeA (bradycardia: −1.32 ± 0.20; $P > 0.05$ and tachycardia: −2.17 ± 0.27; $P > 0.05$) (Fig. 5). A representative recording showing the increase in bradycardic component caused by the microinjection of $CoCl_2$ into the MeA is presented in Fig. 6.

4. Discussion

The acute and reversible pharmacological inhibition of the MeA neurotransmission by local microinjection of $CoCl_2$ enhanced the baroreflex bradycardic response, suggesting that local synapses are involved in the modulation of the parasympathetic component of cardiac baroreflex in conscious rats. The pretreatment with $CoCl_2$ reduces Ca^{2+} pre-synaptic influx leading to an inhibition of neurotransmitters release and consequent synaptic blockade (Kretz, 1984). Therefore, our results suggest that such inhibitory influence of MeA on the baroreflex involves local synapses and not an inhibition of passing fibers.

Analysis of sigmoid curves generated 10 min after $CoCl_2$ microinjection into the MeA indicated a significant increase in the magnitude of the reflex bradycardia caused by the intravenous infusion of phenylephrine, without effect on the tachycardic response to intravenous infusion of SNP. These data suggest that MeA has an inhibitory role on cardiac baroreflex in rats. Moreover, $CoCl_2$ microinjection did not affect BP and HR baselines; a result in accordance with previous reports from our laboratory showing no significant contribution of the MeA in the baseline cardiovascular control (Fortaleza et al., 2009).

Analysis of the baroreflex sigmoid curve showed that bilateral microinjection of $CoCl_2$ into the MeA only increased the slope of the sigmoid curve (G parameter). None of the other parameters analyzed in the sigmoid curve were affected by the treatment with $CoCl_2$, i.e. the lower HR

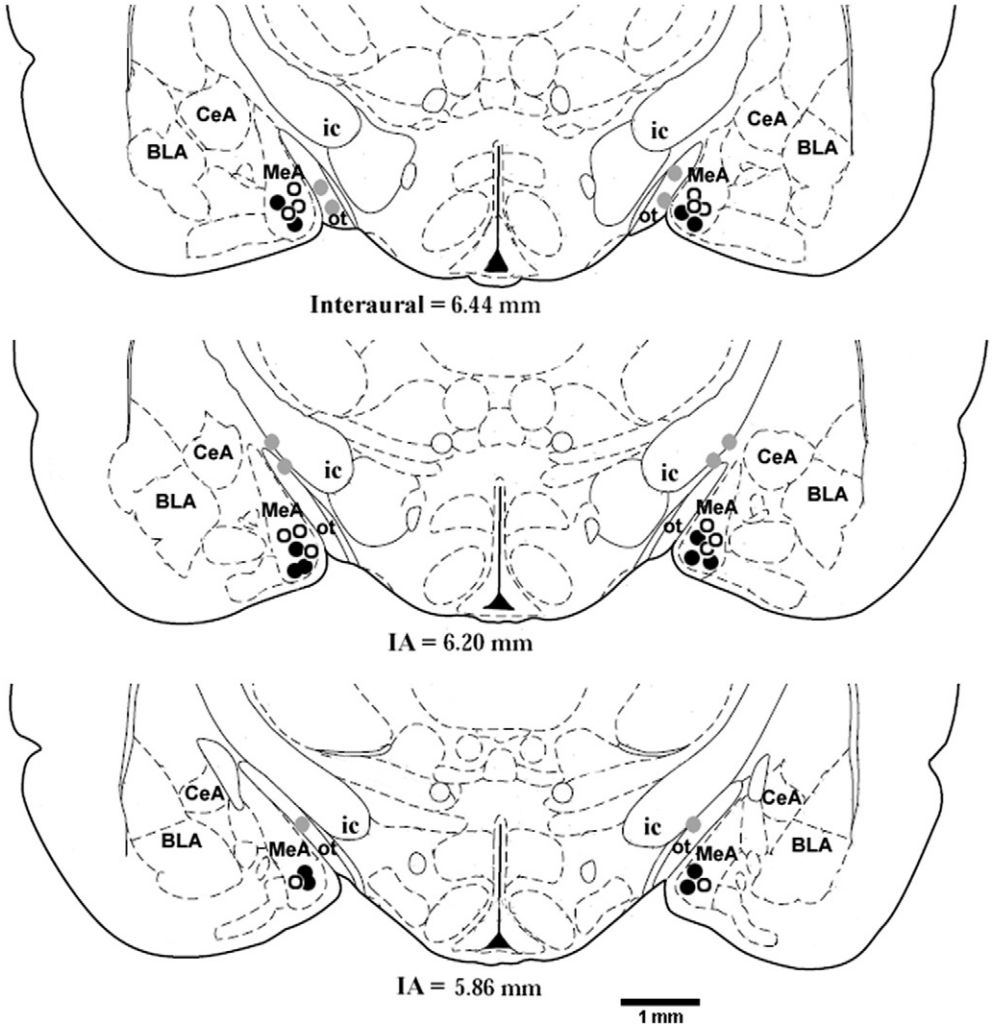

Fig. 2. Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997) indicating injection sites of aCSF (white circles, n = 7) or $CoCl_2$ (black circles, n = 7) in the MeA, and injection sites of $CoCl_2$ in surrounding areas (gray circles, n = 5). ic—internal capsula, ot—optical tract, IA—Interaural coordinate, CeA—central amygdaloid nucleus, BLA—basolateral amygdaloid nucleus.

plateau (P1), upper HR plateau (P2), ΔP (difference between the upper and lower HR plateaus) and BP_{50}. The baroreflex response returned to control values 60 min after $CoCl_2$ administration, indicating reversibility of the synaptic inhibition caused by $CoCl_2$. The present results suggest that reversible inhibition of the MeA neurotransmission with $CoCl_2$ only enhances the bradycardic component of the baroreflex activity in rats.

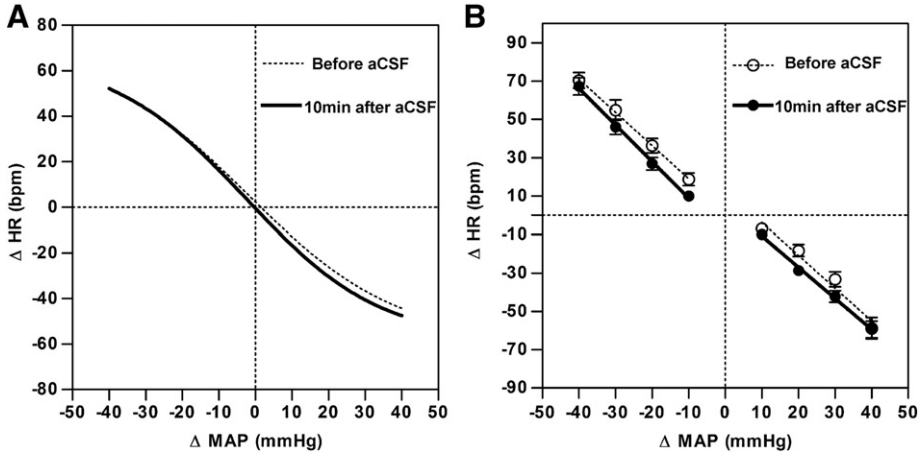

Fig. 3. A) Sigmoid baroreflex curves correlating mean arterial pressure (Δ MAP) and heart rate responses (Δ HR) before ($r^2 = 0.87$) or after ($r^2 = 0.92$) microinjection of artificial cerebrospinal fluid (aCSF) into the MeA. B) Linear regression baroreflex curves correlating mean arterial pressure (Δ MAP) and heart rate responses (Δ HR) before ($r^2 = 0.78$ to tachycardic and $r^2 = 0.78$ to bradycardic) or after microinjection of aCSF in the MeA ($r^2 = 0.86$ to tachycardic and $r^2 = 0.85$ to bradycardic).

Table 1
Parameters derived from the sigmoidal curve generated before and after bilateral microinjection of aCSF (n = 7), 1 mM cobalt into structures surrounding the MeA (n = 5) or 1 mM cobalt into the MeA (n = 7).

	G (bpm/mm Hg)	P1 (bpm)	P2 (bpm)	Range (bpm)
aCSF	$t = 0.40$	$t = 0.08$	$t = 0.44$	$t = 0.18$
Before	−1.10 ± 0.14	−59 ± 7	70 ± 4	129 ± 8
10 min after	−1.19 ± 0.08	−59 ± 4	67 ± 4	127 ± 7
Cobalt surrounding MeA	$t = 0.03$	$t = 0.57$	$t = 0.38$	$t = 0.60$
Before	−1.38 ± 0.12	−67 ± 3	70 ± 6	137 ± 8
10 min after	−1.37 ± 0.09	−63 ± 4	68 ± 4	131 ± 2
Cobalt into the MeA	$F_{(2.20)} = 57.29$	$F_{(2.20)} = 2.51$	$F_{(2.20)} = 0.03$	$F_{(2.20)} = 1.16$
Before	−0.95 ± 0.07	−42 ± 8	76 ± 9	118 ± 14
10 min after	−1.88 ± 0.07*	−66 ± 10	75 ± 11	141 ± 12
60 min after	−1.01 ± 0.06	−44 ± 7	73 ± 8	118 ± 11

Values are means ± SEM. Student's t test was used to compare the values before and after microinjection of aCSF or 1 mM cobalt into structures surrounding the MeA. One-way ANOVA followed by Dunnett's post-test was used to compare the values before, 10 min and 60 min after microinjection of 1 mM cobalt into the MeA. *$P < 0.05$, significant difference from values before and after 1 mM cobalt into the MeA. Slope of the sigmoid curve gain (G); lower HR plateau (P1); upper HR plateau (P2).

To study the baroreflex parasympathetic component, the bradycardic response was analyzed using linear regression analysis. Linear regression analysis indicated that acute inhibition of the MeA neurotransmission enhanced the bradycardic response to MAP increases caused by intravenous infusion of phenylephrine, confirming the hypothesis that the MeA plays an inhibitory role on the cardiac parasympathetic baroreflex modulation. Evidence from power spectral analysis carried out in genetically hypertensive BPH/2J mice indicates that permanent lesion of the MeA increases baroreflex gain due to vagal contribution (Jackson et al., 2014).

Although the present result from the use of $CoCl_2$ points to the MeA as part of the neural brain pathway modulating the bradycardic component of baroreflex, the putative neurotransmitters are not known, and will demand further studies to be identified. Glutamate and GABA are widespread as neurotransmitters in the CNS, and may be considered as putative mediators involved in baroreflex modulation. In the bed nucleus of the stria terminalis (BST), the blockade of glutamatergic NMDA receptors increased the gain of the parasympathetic component of the baroreflex (Alves et al., 2009a), indicating an inhibitory role of the glutamatergic transmission on the parasympathetic component of the baroreflex. The microinjection of glutamate and GABA into the posterodorsal MeA of conscious rats, in a volume of 300 nL, has been reported to decrease maximum baroreflex gain (Neckel et al., 2012), indicating that activation of local glutamatergic and gabaergic receptors could involved in the mediation of the inhibitory role of the MeA on the parasympathetic component of the baroreflex presently evidenced. The MeA noradrenergic neurotransmission should also be considered as a possible candidate to baroreflex modulator. In the BST, the blockade of α1-adrenoceptors increased the gain of the parasympathetic component of the baroreflex (Crestani et al., 2008a), also indicating an inhibitory role of the local BST noradrenergic transmission on the parasympathetic component of the baroreflex. We have previously reported that microinjection of noradrenaline into the MeA evoked pressor and bradycardic responses through acute vasopressin release into circulation (Fortaleza et al., 2011). Vasopressin-mediated cardiovascular responses following noradrenaline microinjection into the MeA are mediated by activation of local α_2-adrenoceptors and magnocellular neurons in the paraventricular and supraoptic nuclei (Fortaleza et al., 2011, 2012a). However, the observation that the magnitude of the bradycardic response evoked by noradrenaline in this area significantly correlates with the magnitude of the pressor response favors the idea of a reflex mechanism. The involvement of the noradrenergic neurotransmission from several brain regions of the central nervous system in the control of baroreflex response has been well documented (Alves et al., 2009b; Crestani et al., 2008a; Hwang et al., 1998; Scopinho et al., 2012). This modulation could either be due to inhibition of baroreceptor input to vagal neurons or to facilitation of an inhibitory drive to these neurons, thus enhancing parasympathetic response to blood pressure increase. Alternatively, the bradycardic response caused by activation of noradrenergic receptors could be due to a direct influence of the MeA on vagal activity, without interference with baroreflex modulation. Further experiments are necessary to clarify this issue. Although central glutamatergic, gabaergic, and noradrenergic pathways seem to play a role in baroreflex activity, its involvement in baroreflex modulation by the MeA yet has to be evaluated.

There is evidence showing that dense neuronal connections between the MeA and brainstem and supramedullary areas such as the

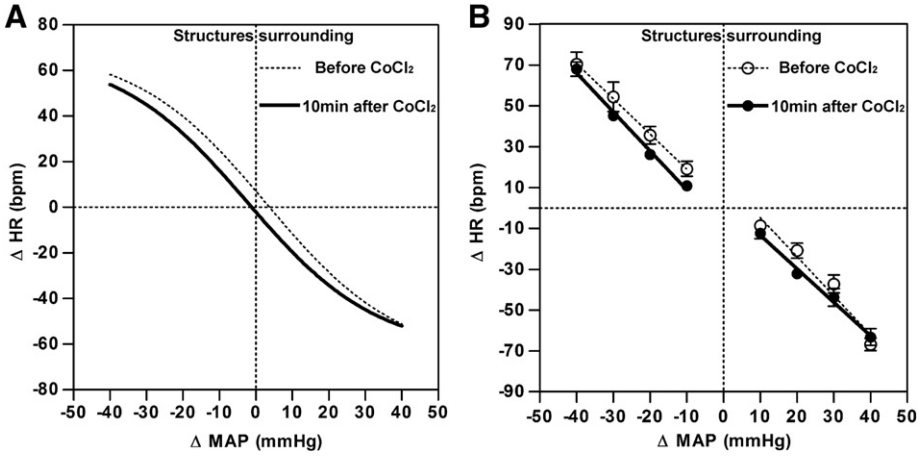

Fig. 4. A) Sigmoid baroreflex curves correlating mean arterial pressure (Δ MAP) and heart rate responses (Δ HR) before ($r^2 = 0.92$) or after the microinjection of $CoCl_2$ into structures surrounding the MeA ($r^2 = 0.96$). B) Linear regression curves for baroreflex, correlating mean arterial pressure (Δ MAP) and heart rate responses (Δ HR) before ($r^2 = 0.76$ to tachycardic and $r^2 = 0.87$ to bradycardic) or after microinjection of $CoCl_2$ in structures surrounding the MeA ($r^2 = 0.95$ to tachycardic and $r^2 = 0.87$ to bradycardic).

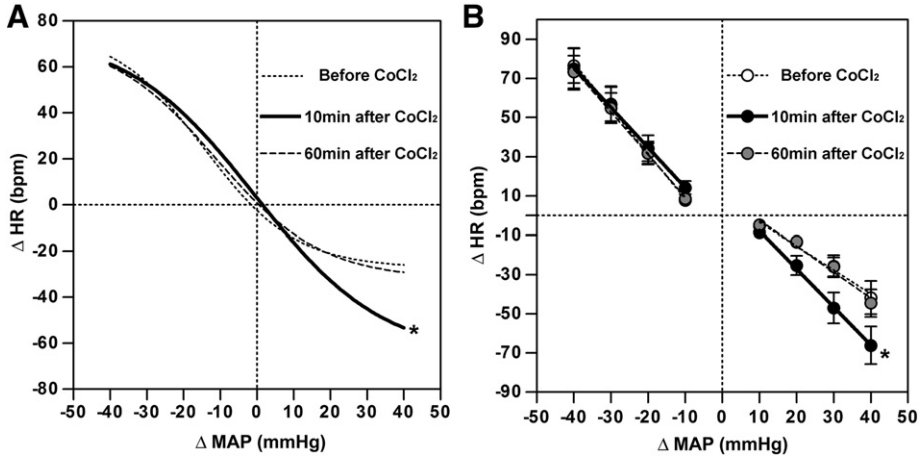

Fig. 5. A) Sigmoid baroreflex curves correlating mean arterial pressure increase (Δ MAP) and heart rate response (Δ HR) before ($r^2 = 0.82$), 10 min ($r^2 = 0.83$) or 60 min ($r^2 = 0.82$) after bilateral microinjection of 1 mM of $CoCl_2$ into the MeA (n = 7). B) Linear regression curve correlating mean arterial pressure increase (Δ MAP) and bradycardic response (Δ HR) before ($r^2 = 0.70$), 10 min ($r^2 = 0.58$) or 60 min ($r^2 = 0.68$) after bilateral microinjection of 1 mM of $CoCl_2$ into the MeA. Linear regression curve correlating mean arterial pressure decrease (Δ MAP) and tachycardic response (Δ HR) before ($r^2 = 0.52$), 10 min ($r^2 = 0.63$) or 60 min ($r^2 = 0.60$) after bilateral microinjection of 1 mM of $CoCl_2$ into the MeA (*) $P < 0.05$, significantly different between sigmoid curves or linear regressions parameters $CoCl_2$ and aCSF, one-way ANOVA.

hypothalamus and the BST (Dampney, 1994; Fortaleza et al., 2011, 2012a; Ohta et al., 1991). The role of medullary structures in the baroreflex is well documented (Michelini, 2007; Spyer, 1981; Sved and Gordon, 1994). In addition, the BST is a functionally heterogenous component of the extended amygdala that has been implicated in inhibitory actions on the cardiac baroreflex parasympathetic component, as well as in the modulation of cardiovascular responses during stressful situations (Crestani et al., 2006, 2007). It has been reported that the MeA modulates autonomic responses to stressful stimuli (Canteras et al., 1995; Chen and Herbert, 1995; Cullinan et al., 1995; Dayas et al., 2001a; Fortaleza et al., 2012b; Fortaleza et al., 2009; Kubo et al., 2004), and that the baroreflex parasympathetic component is suppressed during stress (Nosaka, 1996). Therefore, we suggest that the MeA could be modulating the baroreflex parasympathetic component during defensive situations.

Baroreflex inhibition was also reported to involve other areas of the limbic system, such as the dorsolateral periaqueductal gray matter (Nosaka et al., 1993; Pelosi et al., 2007), the medial prefrontal cortex (Resstel and Correa, 2006; Resstel et al., 2004), lateral septal area (Scopinho et al., 2007, 2012) and the diagonal band of Broca (Crestani et al., 2008b). These areas are connected to the MeA (Bienkowski et al., 2013; Canteras et al., 1995), suggesting the existence of an interrelation between them in baroreflex modulation under aversive conditions. Moreover, the MeA plays an inhibitory role on the cardiac component of restraint-evoked cardiovascular responses in rats (Fortaleza et al., 2009). These results support the idea that the MeA can modulate the baroreflex cardiac component during stress situations.

In summary, the present results showing that acute synaptic inhibition of the MeA, caused by the microinjection of $CoCl_2$, enhanced cardiac baroreflex bradycardic responses suggest that local synapses in that area are involved in the modulation of bradycardic reflex magnitude in response to MAP increases caused by phenylephrine infusion, suggesting a tonic inhibitory role of the MeA on the bradycardic baroreflex component. Results also indicate that MeA inhibition does not affect the tachycardic baroreflex response to MAP decreases, in normotensive conscious rats.

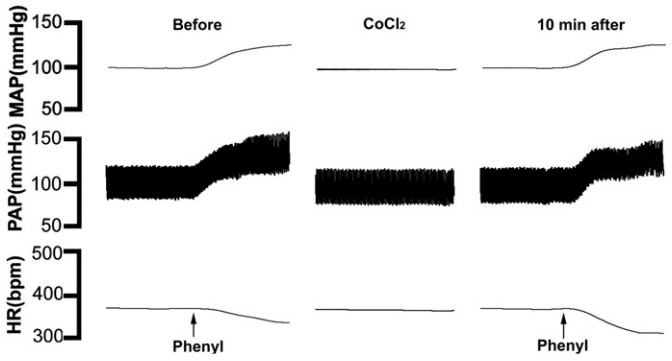

Fig. 6. Recordings of one $CoCl_2$-treated animal illustrating the bradycardic reflex in response to blood pressure increase caused by intravenous phenylephrine infusion before and 10 min after the bilateral microinjection of $CoCl_2$ (1 mM) into the MeA.

5. Conclusions

The microinjection of $CoCl_2$ into MeA enhanced cardiac baroreflex bradycardic responses suggest that local synapses in that area are involved in the modulation of bradycardic reflex magnitude in response to MAP increases caused by phenylephrine infusion, suggesting a tonic inhibitory role of the MeA on the bradycardic baroreflex component.

Acknowledgments

The authors wish to thank I.A.C. Fortunato, I.I.B. Aguiar and S.S. Guilhaume for technical help. Grants: Fortaleza has a FAPESP postdoctoral fellowship (2012/18556-2); Ferreira-Junior is supported by FAPESP PhD fellowship (2011/19494-8) and Lagatta of PhD fellowship from CAPES (PROEX: 461/2009). The present research was supported by grants from the CNPq (474477/2013-4), FAPESP and FAEPA.

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